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Evaluation of Atmospheric Water Generation Technology: Microbial Water Quality

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Evaluation of Atmospheric Water Generation Technology: Microbial Water Quality

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Notice/Disclaimer Statement

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Abstract

Atmospheric water generators (AWGs) have the potential to expand the availability of water during shortages, contamination events and interruptions of service. Given the nature of atmospheric water generation (i.e., condensation of atmospheric water vapor), high quality produced water is generally anticipated; however, it may not be safe for human consumption. Concentrating large volumes of air can simultaneously concentrate contaminants, and microbial growth in plumbing and stored water is possible. An initial review of water quality data provided by Watergen USA (hereafter referred as Watergen), a manufacturer of AWG technology, confirmed both the generally high quality of produced water (e.g., no elemental analyses above current EPA primary or secondary drinking water standards), but an elevated level of overall microbial numbers (i.e, heterotrophic bacteria). Indicators of fecal contamination (e.g., fecal coliforms*, E. coli)* were not detected. The primary microorganisms of human health concern are opportunistic pathogens, such as *Legionella* spp. and *Mycobacterium* spp., that are commonly associated with drinking water infrastructure. Detection of these organisms, particularly *L. pneumophila* serogroup 1 and *M. avium*, would constitute a potential health concern. The objective of this study was to evaluate the microbial quality of untreated condensate and produced (treated) water from a commercial AWG unit (Watergen GEN-350) during the three-month study. Opportunistic pathogens were not detected in weekly samples collected; however, high levels of heterotrophic bacteria, detected using heterotrophic plate counts (HPC) of treated water, indicate inadequate disinfection and/or microbial regrowth following treatment. The presence of heterotrophic bacteria do not represent a human health risk, but suggest microbial instability and conditions favorable for microbial growth. It is therefore recommended that chlorination or ozonation be included as an additional unit process in the GEN-350 treatment train. While not directly transferrable to other AWG systems, results of this work emphasize that atmospheric condensate is not sterile and should be treated adequately prior to potable use. This report covers a period from February 28, 2018 to September 6, 2018 and work was completed as of September 28, 2018.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

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Acronyms and Abbreviations

AWG	Atmospheric water generator
BLQ	Below limit of quantification
CBI	Confidential business information
CFU	Colony forming unit
CRADA	Cooperative research and development agreement
Ct	Cycle threshold
EPA	Environmental Protection Agency
FB	Field blank
HDPE	High density polyethylene
HPC	Heterotrophic plate counts
ICP	Inductively coupled plasma
IPC	Internal positive control
LOQ	Limit of quantification
MB	Method blank
MCL	Maximum contaminant level
MRDL	Maximum residual disinfectant level
MOH	Ministry of Health
NERL	National Exposure Research Laboratory
NTC	No template control
NTU	Nephelometric turbidity units
ORD	Office of Research and Development
QA	Quality assurance
QAPP	Quality Assurance Project Plan
qPCR	Quantitative polymerase chain reaction
SED	Systems Exposure Division
SM	Standard Methods for the Examination of Water and Wastewater
SWTR	Surface Water Treatment Rule
T&E	EPA Test and Evaluation Facility
TDS	Total dissolved solids
TT	Treatment technique
UV	Ultraviolet

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1.0 Introduction

Atmospheric water generators (AWGs) produce potable water from ambient air. These units range from home-based units that can produce 1 to 20 liters of water per day to commercial-scale units capable of 1,000 to over 10,000 liters per day. Water production rates are highly dependent upon the amount of water vapor in the atmosphere (*i.e.*, humidity), and temperature of the air. The most commonly used AWG systems employ condenser and cooling coil technology to pull moisture from the air in the same way a household dehumidifier does. Significant quantities of energy can be required to operate these condenser and fan systems, but recent technological advancements have substantially improved the energy-water ratio, which increases the feasibility of using these systems to provide clean water in a cost-effective way.

EPA has explored the feasibility of AWG systems for different scenarios in recent years. For instance, EPA's Office of Land and Emergency Management examined the application of AWGs to supplement or provide drinking water during Superfund response actions. Although they found the energy cost of AWG water production to be higher than the cost of obtaining water from a public water supply, they also noted that AWG water production has a lower cost than providing bottled water in an emergency or alternative scenario where a public water supply is not available. Recent high-profile natural disasters, such as Hurricane Harvey, and public water infrastructure failures, such as those that occurred in Flint, Michigan, have increased the interest in AWG technology as both emergency and longer-term supply solutions.

In August of 2017, EPA's Office of Research and Development (ORD) announced a Cooperative Research and Development Agreement (CRADA) developed to further advance the science of AWG, and assess its potential as a water production and supply solution for different scenarios. In January 2018, EPA signed a CRADA with Watergen, a corporation developing cutting-edge AWG technology, to evaluate their GEN-350 system (http://water-gen.com/). The GEN-350 is capable of generating nearly 600 liters of water per day in optimal temperature and humidity conditions. Collected water is periodically recirculated through a treatment system consisting of a sediment filter, carbon filter, and ultraviolet (UV) disinfection. It is then delivered on-demand via an integrated tap following mineralization and polish filtration. A test unit was shipped to EPA lab facilities for collaborative research purposes.

This report covers the results of an overall assessment of the quality of water produced by the AWG. As a first step, EPA-ORD agreed to review water quality data from the Watergen system provided by a 3rd party laboratory. The purpose of this effort was to review the data with respect to EPA drinking water standards, and focus subsequent sampling of the Watergen system by ORD. This evaluation confirmed that the microbiological quality of atmospherically generated water was of greatest concern, and a long term (3 month) study was conducted to determine the potential for growth of opportunistic microbial pathogens (*Legionella, Mycobacterium*) commonly associated with water distribution systems.

2.0 Assessment of Third Party Water Quality Data

On October 13, 2017, Watergen provided EPA-ORD commercial laboratory test results on water quality produced from a GEN-350 unit identical to the unit used for testing under the CRADA with EPA. The test results were performed on product water samples from the GEN-350 and consisted of two elemental analyses (conducted on July 12, 2017 and July 16, 2017), a microbiological analysis (conducted July 17, 2017), and an inorganic and microbiological analysis (conducted July 23, 2017). All testing was performed by AMINOLAB (AMINOLAB LTD. Analytical Laboratory Services Weizmann Science Park 1, Pinhas Sapir St. P.O.B. 4074 Ness Ziona. 70400 Israel). The laboratory report indicates that the samples were collected by "The Customer" (presumed to be Watergen representatives). The summaries provided below on the results have not undergone a rigorous quality assurance/quality control review by EPA nor were they performed under an EPA Quality Assurance Project Plan. The following sections simply describe the results provided to EPA-ORD by Watergen. The specific data are not reported as they are considered confidential business information (CBI); the following is the ORD review of the data in with respect to EPA drinking water standards.

For the water samples tested for elements, no results were above current EPA primary or secondary drinking water standards. Results from the laboratory report do not clarify whether the two "Metal Scans" were performed on the same sample or from two separate samples from the GEN-350 unit. The method used for the analyses was from Standard Methods for the Examination of Water and Wastewater (SM) (prepared and published jointly by the American Public Health Association, American Water Works Association, and the Water Environment Federation), which are commonly used consensus standard methods widely used and accepted in the water industry. The laboratory report also states that AMINOLAB is accredited under ISO/IEC 17025 for standard operating procedures and is accredited for analysis for most of the analytes listed in Table 1 (denoted by *). All elemental analyses were conducted by SM-3120B-Metals by Plasma Emission Spectroscopy-Inductively Coupled Plasma (ICP) Method. After review of the laboratory report, EPA-ORD contacted Watergen to clarify whether AMINOLAB analyzed the samples for "total" or "dissolved" metals. Watergen stated that the analyses were performed for "total" metals, however the digestion method used for the samples was not provided in the laboratory report.

Table 1. Elements analyzed for product water samples from the Watergen GEN-350 unit

Element	Symbol	EPA National Primary Drinking Water Standard MCL or TT[1] (mg/L)	EPA National Secondary Drinking Water Standard (non-enforceable guideline) (mg/L)
Silver*	Ag	NA	0.10
Aluminum*	Al	NA	0.05 to 0.2
Arsenic*	As	0.010	NA
Boron*	B	NA	NA
Barium*	Ba	2	NA
Beryllium*	Be	0.004	NA
Calcium*	Ca	NA	NA
Cadmium*	Cd	0.005	NA
Cobalt*	Co	NA	NA

Chromium*	Cr	0.1 (total Cr)	NA
Copper*	Cu	1.3 (TT action level)	1.0
Iron*	Fe	NA	0.3
Potassium*	K	NA	NA
Lithium*	Li	NA	NA
Magnesium*	Mg	NA	NA
Manganese*	Mn	NA	0.05
Molybdenum*	Mo	NA	NA
Sodium*	Na	NA	NA
Nickel*	Ni	NA	NA
Phosphorus	P	NA	NA
Lead*	Pb	0.015 (TT action level)	NA
Sulfur*	S	NA	NA[2]
Selenium*	Se	0.05	NA
Tin	Sn	NA	NA
Strontium	Sr	NA	NA
Titanium	Ti	NA	NA
Vanadium	V	NA	NA
Zinc	Zn	NA	5

[1] MCL = Maximum Contaminant Level. TT = Treatment Technique (a required process intended to reduce the contaminant level in drinking water).
[2] A secondary DW standard exists for sulfate (250 mg/L).
*Analytes for which AMINOLAB is accredited for under ISO/IEC 17025.

Additional analyses performed by AMINOLAB consisted of pH, conductivity, turbidity, free chlorine, and total chlorine measurements. These measurements are listed in the lab report as "field measurements". The field measurements were taken according to the Israeli Ministry of Health's sampling guidelines and AMINOLAB was accredited to perform these analyses under ISO/IEC 17025 at the time of testing. Table 2 provides information on applicable EPA standards for the measurements on the Watergen GEN-350 product water.

Table 2. List of measurements for additional analysis of product water samples from the Watergen GEN-350 unit

Analysis	EPA National Primary Drinking Water Standard MCL or TT or Surface Water Treatment Rule requirement	EPA National Secondary Drinking Water Standard (non-enforceable guideline)
pH	NA	6.5-8.5 (pH units)
Conductivity	NA	NA
Turbidity	5 NTU[1]	NA
Free Chlorine	MRDL = 4.0[2]	NA
Total Chlorine	MRDL = 4.0[2]	NA

[1] NTU = Nephelometric turbidity units. Under the surface water treatment rules, at no time can turbidity go above this limit (5 NTU). There are additional turbidity requirements for systems that use filtration in their treatment process (see https://www.epa.gov/dwreginfo/surface-water-treatment-rules).
[2] MRDL = Maximum residual disinfectant level. Measured as Cl_2. The USEPA MRDL of 4.0 mg/L is the highest level of a disinfectant allowed in drinking water. Under EPA's Surface Water Treatment and

Groundwater Rules, a minimum chlorine disinfectant residual of 0.2 mg/L is required for most systems at the entry point to the drinking water distribution system and detectable throughout.

It should be noted that, within the lab report provided by Watergen, the Israeli Ministry of Health (MOH) Requirements for free chlorine in drinking water range from 0.1 mg/L to 0.5 mg/L. Free and total chlorine results from the GEN-350 product water were less than 0.1 mg/L indicating that the product water tested did not have a chlorine residual that met Israeli MOH requirements.

EPA surface water treatment rules contain treatment technique requirements for systems that exceed specified turbidity levels (as noted in Table 2). The Watergen GEN-350 unit contains filtration components that could be classified as "alternative filtration" within the context of U.S. drinking water standards which apply to surface water sources (though this report recognizes that the source water for the Watergen unit would generally not be considered as surface water). Under the Long Term 1 Enhanced Surface Water Treatment Rule, alternative filtration (e.g. membranes, cartridges, or other), turbidity requirements are set by the state, but must not exceed 1 NTU in 95% percent of samples (taken at least every 4 hours). If a water system serves populations <500, turbidity monitoring frequency can be decreased to one sample per day, if approved by EPA. According to the laboratory report, the Israeli MOH requires that the NTU value be below 1 for drinking water. The turbidity results in the provided lab report are below 1 NTU.

Results provided in the lab report for one of two samples included Total Count (Heterotrophic Plate Count-HPC), coliforms, fecal coliforms, and *Streptococcus fecalis*/*Enterococcus* Group analyses. All microbial analyses were performed using widely accepted methods from SM: Total Count/HPC, SM-9215B-Heterotrophic Plate Count-Pour Plate Method, SM-9222B-Membrane Filter Technique for Members of the Coliform Group-Standard Total Coliform Membrane Filter Procedure, SM-9222D-Membrane Filter Technique for Members of the Coliform Group-Fecal Coliform Membrane Filter Procedure, and SM-9230C-Fecal Streptococcus and Enterococcus Groups-Membrane Filter Techniques were used for Total Count, Coliforms, Fecal Coliforms and *Streptococcus fecalis*/*Enterococcus* groups, respectively. At the time of testing, the laboratory report states that AMINOLAB was accredited to perform these analyses under ISO/IEC 17025 with standard operating procedures and recognized to perform these analyses by the Israeli Ministry of Health.

The second sample included results for the same microbial contaminants as the first sample, but also included analysis for *Escherichia coli* by SM-9222G-Membrane Filter Technique for Members of the Coliform Group-MF Partition Procedures.

Table 3. List of measurements for microbial analysis for product water samples from the Watergen GEN-350 unit

Analysis	EPA National Primary Drinking Water Standard MCL or TT or Surface Water Treatment Rule requirement	EPA National Secondary Drinking Water Standard (non-enforceable guideline)
Total Heterotrophic Bacteria (using HPC method)	500 CFU/mL[1]	NA
Coliforms	5.0%[2]	NA
Fecal Coliforms	NA	NA

Streptococcus faecalis/*Enterococcus* Group	NA	NA
E. coli	See[2]	NA

[1] CFU = Colony forming units. Under EPA's surface water treatment rules, systems may use HPC measurements as an alternative indicator of the presence of a disinfectant residual. Systems in exceedance of the 500 CFU/mL limit in more than 5% of samples each month for two consecutive months are in violation of the regulatory requirements.

[2] No more than 5.0% of samples can be total coliform-positive in a month. If a system exceeds this level, they must conduct an assessment of the system. For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive in a month. Samples that are positive for total coliforms must be tested for *Escherichia coli*. The system must also collect repeat samples (see https://www.epa.gov/dwreginfo/revised-total-coliform-rule-and-total-coliform-rule for details).

The lab report results from AMINOLAB reported no detections (<1 CFU/100 mL) for coliforms, fecal coliforms and *Streptococcus fecalis*/*Enterococcus* group for samples one and two. Sample two was also reported as a non-detect (<1 CFU/100 mL) for *E. coli*. Heterotrophic plate count results (listed as Total Counts in the lab report) exceeded the EPA National Primary Drinking Water Standard value in both samples (>500 CFU/mL).

The two main issues from the laboratory report from Watergen on product water from the GEN-350 unit are: 1) absence of a disinfectant residual and 2) elevated HPC values for the product water. In addition to adding a disinfectant to Watergen GEN-350 product water, it is also recommended that product water turbidity be monitored every 4 hours or once daily (with state approval) during water production. The elevated HPC levels suggest that the extent and nature of microbial growth within the product water should be quantified during longer term operation; results of this study are presented below.

3.0 AWG Testing - Methods

Following a 1-month period of acclimation, the GEN-350 was sampled on an approximately weekly basis for 3 months to assess the microbial water quality of both raw (untreated) and treated product water. These analyses included HPC and quantitative polymerase chain reaction (qPCR) for opportunistic pathogens *L. pneumophila* serogroup 1, *M. avium*, and *M. intracellulare*. In addition, pH and conductivity of the treated water were monitored to ensure that they remained within manufacturer operating range. To maintain production and sampling of fresh water, the GEN-350 was routinely drained on Mondays and Thursdays or when the unit was entirely full; microbial samples were typically collected on Wednesdays and pH and conductivity measurements were typically performed on Mondays prior to draining. The GEN-350 was operated indoors in a heated but not air-conditioned facility.

During each microbial sampling event (n=10), the unit's ambient temperature water tap was disinfected using 70% isopropyl alcohol wipes prior to collection of 15L treated water into a 20L HDPE carboy. Additionally, up to 2L untreated condensate (depending on quantity available) was manually collected into 1L HDPE bottles by removing the plastic quick-release plug from the bottom of the stainless-steel canister that collects water directly from the evaporators. All sample containers were cleaned using a 10% bleach solution, neutralized, and autoclaved (15 min at 15 psi and 121°C) prior to use. During 3 sampling events (beginning, middle, and end of study), field blanks were collected by pouring 1L molecular grade water

into the 20L HDPE carboy and back into a 1L HDPE collection bottle.

Upon receipt at the laboratory, samples were mixed by shaking and subsampled for various analyses. For the treated water, 1L subsamples were poured into clean, autoclaved 1L HDPE containers for each of the 4 analyses (HPC and 3 qPCR targets); 10L of the remainder was reserved for future metagenomic microbial community analysis (not included in this report). For the raw (untreated) water, the sample was either divided in half (7 samples) into clean, autoclaved 1L HDPE containers or, if the volume was ≤0.5L, the entire volume was used for either HPC and qPCR or metagenomics, alternating between the analyses sets (3 samples).

DNA was extracted for qPCR as described by Beumer et al. (2015). Briefly, subsamples were vacuum filtered; bead-beaten to disrupt bacterial cells; treated to remove proteins, RNA, and other cellular debris; and precipitated by alcohol prior to DNA resuspension in 50µL molecular grade water. Method blanks were prepared during each batch of sample processing by substituting 1L molecular grade water for experimental samples. qPCR was performed in triplicate on the Applied Biosystems StepOnePlus instrument using 5uL (for *L. pneumophila*) or 10uL (for *M. avian* and *M. intracellulare*) extracted DNA and primer/probe sets reported by Merault et al. (2011) and Chern et al. (2015), respectively. No template controls (NTCs) were maintained on each qPCR plate, and PCR inhibition was monitored using an exogenous internal positive control (IPC) added to each reaction. If applicable, sample concentrations were determined by interpolation of cycle threshold (Ct) values against a master standard curve prepared from 10-fold serial dilutions of quantified genomic DNA included on each plate. Limits of quantification (LOQs) were based on the lowest-concentration quantifiable standard (10 gene copies/PCR reaction for each target) and were 100 gene copies/L sample for *L. pneumophila* and 50 gene copies/L sample for *M. avian* and *M. intracellulare*. Sample PCR inhibition was identified by IPC Ct>40 (*i.e.*, IPC not measurable during the 40-cycle reaction).

HPC methods followed Standard Method 9215C (APHA 2017), using R2A agar and 7-day incubation at 25°C. Multiple dilutions of each sample, typically ranging from undiluted to 1:100 sterile molecular biology-grade water, were analyzed in duplicate. Sample concentrations were determined by averaging each replicate containing ≥30 and ≤500 colony forming units (CFU) per plate. Negative controls, which included filtered, sterile molecular biology-grade water, were included with each processing batch. pH and conductivity were measured using a YSI 556 Multi-Probe System following manufacturer instructions. Monthly calibrations were performed and check standards analyzed with each weekly sample.

4.0 AWG Testing - Results and Discussion

Opportunistic pathogens *L. pneumophila* serogroup 1, *M. avium*, and *M. intracellulare* were not detected in any raw or treated water samples (Table 1). However, the last four treated water samples were PCR-inhibited and pathogen detection was therefore undetermined. The source of this inhibition remains unknown; however, fine gray debris were noted when filtering the treated water. All qPCR method blanks and NTCs were negative; one field blank was positive for *M. avium,* but this finding did not impact study results (corresponding experimental samples were negative). All standards within the range of quantification (10^1–10^6 copies/PCR reaction) were positive; infrequent cases of nonlinearity among serial dilutions did not impact negative sample determinations. While failure to detect opportunistic pathogens in raw and treated waters suggests low health risk from these organisms during the study period, high heterotrophic bacteria counts (see below) indicate microbial instability and therefore the potential for their colonization. *L. pneumophila* has been previously detected in untreated condensate from home air conditioning units, which are functionally similarly to AWG (Alipour et al. 2015).

Table 4. qPCR results for opportunistic pathogens

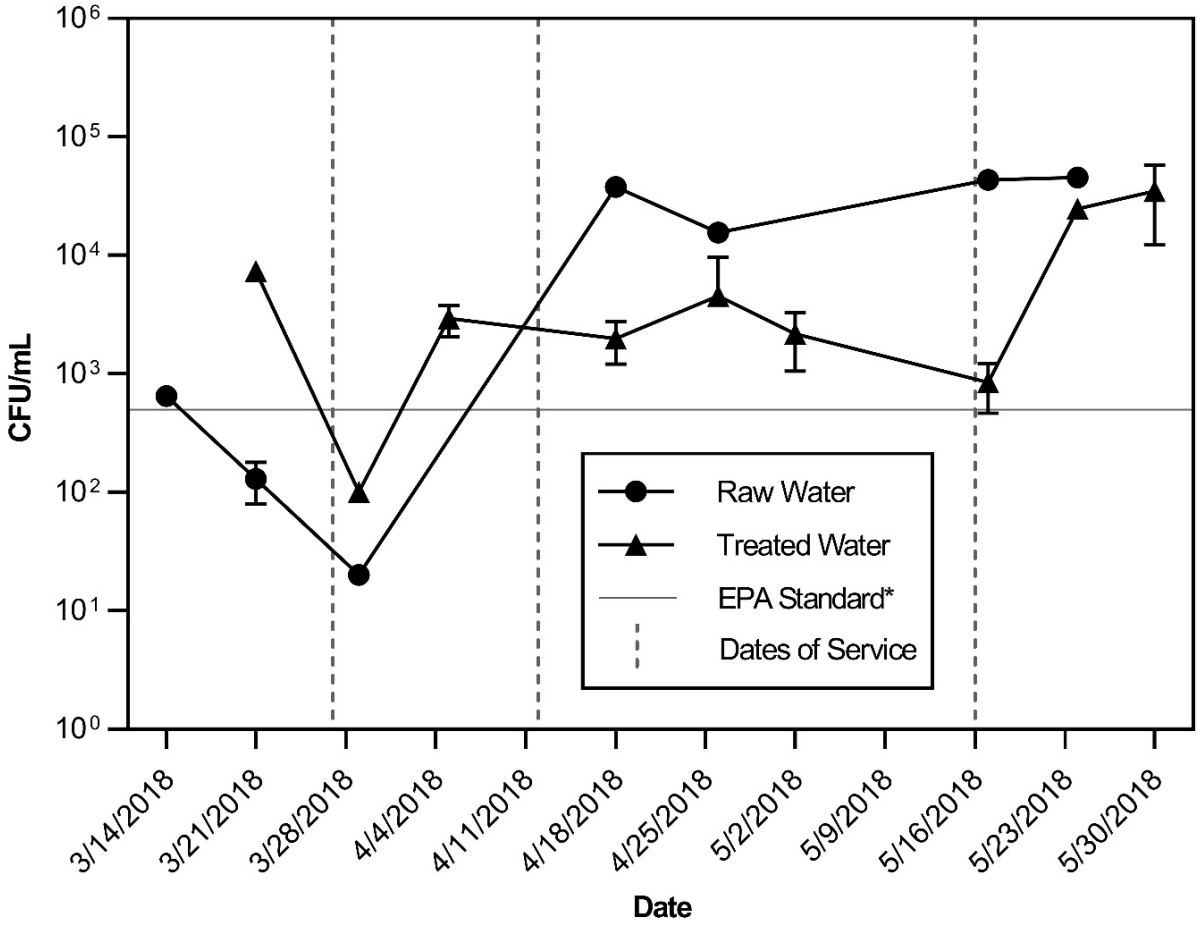
	Collection Date	*M. avium*	*M. intracellulare*	*L. pneumophila* serogroup 1
Raw Water	3/14/2018	BLQ[1]	BLQ	BLQ
	3/21/2018	BLQ	BLQ	BLQ
	3/29/2018	BLQ	BLQ	BLQ
	4/18/2018	BLQ	BLQ	BLQ
	4/26/2018	BLQ	BLQ	BLQ
	5/17/2018	BLQ	BLQ	BLQ
	5/24/2018	BLQ	BLQ	BLQ
	5/30/2018	BLQ	BLQ	BLQ
Treated Water	3/14/2018	BLQ	BLQ	BLQ
	3/21/2018	BLQ	BLQ	BLQ
	3/29/2018	BLQ	BLQ	BLQ
	4/5/2018	BLQ	BLQ	BLQ
	4/18/2018	BLQ	BLQ	BLQ
	4/26/2018	BLQ	BLQ	BLQ
	5/2/2018	Undetermined[2]	Undetermined	Undetermined
	5/17/2018	Undetermined	Undetermined	Undetermined
	5/24/2018	Undetermined	Undetermined	Undetermined
	5/30/2018	Undetermined	Undetermined	Undetermined

[1] BLQ = Below limit of quantification: <50 gene copies/L for *M. avium* and *M. intracellulare*; <100 gene copies/L for *L. pneumophila* serogroup 1.

[2] Undetermined due to sample inhibition of PCR reaction.

HPC results are shown in Figure 1. Treated water exceeded the EPA alternative disinfectant residual limit (≤500 CFU/mL) for drinking water systems using surface water in 9/10 samples. This standard does not apply to AWG source waters, which lack the fecal influences associated with surface water (EPA 2018). However, while high levels of heterotrophic bacteria are not directly associated with human health risk, it does indicate microbial instability of the water and is evidence of inadequate

disinfection and/or regrowth of bacteria following treatment (Bartram et al. 2003). Previous studies of commercial air handing units have demonstrated both low (10^0–10^1 most probable number/mL; Glawe et al. 2016) and high (10^5–10^7 CFU/mL; Hugenholtz and Fuerst 1992) HPC in untreated atmospheric condensate collections, suggesting large variability in the quality of these waters. No growth was observed for field blanks, and two method blanks with a single colony each did not impact sample interpretation (concentrations negligible compared to corresponding samples). Routine service, including filter replacement and chlorine disinfection of the unit, did not reliably improve treated water HPC.



*Under EPA's surface water treatment rules, systems may use HPC measurements as an alternative indicator of the presence of a disinfectant residual. Systems in exceedance of the 500 CFU/mL limit in more than 5% of samples each month for two consecutive months are in violation of the regulatory requirements

**Not shown are two samples for which all plates were too numerous to count (TNTC): 3/14/2018 Treated Water was >5,000 CFU/mL; 5/30/2018 Raw Water was >50,000 CFU/mL

Figure 1. HPC results

pH of the treated water ranged 6.01–7.78 (Table 2), within or near the operating range reported by Watergen (6.5–8.5). Three samples were below the non-enforceable EPA secondary standard for pH (6.5–8.5); low pH may result in bitter metallic taste and corrosion, but does not present a health concern

(EPA 2018). Conductivity of treated samples was very low (18–114 µS/cm), frequently below Watergen estimates (approximately 78-313 µS/cm based on reported 50-200 mg/L total dissolved solids (TDS)). The EPA secondary standard for TDS is <500 mg/L (approximately 782 µS/cm); low conductivity does not present a health concern.

Table 5. pH and conductivity of treated water

Collection Date	pH	Conductivity (µS/cm)
3/15/2018	6.69	25
3/19/2018	6.01	33
3/26/2018	6.22	37
4/2/2018	7.19	97
4/9/2018	7.50	82
4/17/2018	7.78	45
4/24/2018	6.79	61
4/30/2018	7.61	74
5/7/2018	7.52	71
5/14/2018	7.11	94
5/21/2018	7.31	77
5/29/2018	7.01	83
6/4/2018	7.02	68

5.0 AWG Testing - Conclusions and Recommendations

An initial review of water quality data provided by Watergen, confirmed the generally high quality of atmospherically generated water (e.g., no elemental analyses above current EPA primary or secondary drinking water standards), but an elevated level of overall microbial numbers (i.e, heterotrophic plate counts). Subsequent testing by EPA-ORD focused on potential microbiological risks, and found no detections of opportunistic pathogens in the generated water during 3 months of continuous operation. The high heterotrophic bacteria levels of both raw and treated AWG waters indicate that they are suitable environments for microbial growth. This also suggests that either the treatment system is inadequate for bacterial removal and/or that significant regrowth occurs post-treatment in distribution lines. These concerns could be addressed by incorporating chlorination or ozonation as an additional disinfection process in the treatment system and ensuring that a residual concentration of the disinfectant is maintained throughout the distribution line. The observed visible debris and associated PCR inhibition at the end of the study should be examined further to diagnose causes and assess their potential impact on drinking water quality.

6.0 References

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Appendix A - Quality Assurance and Quality Control

AWG testing was performed under the NERL Quality Assurance Project Plan (QAPP) "Evaluating the Microbial Quality of Atmospheric Water Generator Condensate" (D-SED-0031412-QP-1-0, effective date 2/28/18); third party data provided by Watergen was not subject to EPA quality assurance (QA) procedures and was not collected under an approved QAPP. Audits of data quality (ADQ) for EPA AWG testing were conducted on 8/2/2018–8/3/2018 for microbial analyses and on 8/24/2018 for pH and conductivity measurements. All findings were addressed following corrective actions approved by the NERL/SED QA Manager and did not impact interpretation of results.

Tables A1–A4 summarize the results of QA controls during AWG testing by EPA. Observed HPC growth on two method blanks (3/14/2018 and 5/2/2018; Table A1) consisted of a single colony each and had a negligible impact on corresponding sample measurements (may be overestimated by 10 CFU/mL, representing <2% difference in sample concentrations). Detection of *M. intracellulare* in the 4/18/2018 field blank (Table A1) was at an average Ct of 39.06, close to the Ct <40 cutoff for positive determination and representing approximately one gene copy; study results were unimpacted since the target was not detected in any experimental samples. qPCR standard curves (10^6–10^0 copies in Table A2) demonstrated successful PCR amplification of control DNA and quantification limits of 10^1 copies (lowest concentration standards with reliable detection), but were not used for quantification of samples due to non-detection of the targets; all qPCR NTCs were successfully negative. Results of the exogenous IPC (Table A3) indicate that all raw water samples and the first six treated water samples (3/14/2018–4/26/2018) did not inhibit the PCR (IPC within acceptable range), yet the final four treated water samples (5/2/2018–5/30/2018) completely inhibited the reaction (no detection of the IPC), rendering associated pathogen determinations inconclusive. All check standards for pH and conductivity measurements (Table A4) were within acceptable ranges.

Table A1. Method blank and field blank results for microbial analyses; target concentration is zero CFU/mL or below limit of quantification (BLQ)

Collection Date	Blank Type[1]	HPC (CFU/mL)	*M. avium* (gene copies/L)	*M. intracellulare* (gene copies/L)	*L. pneumophila* serogroup 1 (gene copies/L)
3/14/2018	MB	10	BLQ[2]	BLQ	BLQ
3/14/2018	FB	0	BLQ	BLQ	BLQ
3/21/2018	MB	0	BLQ	BLQ	BLQ
3/27/2018	MB	0	BLQ	BLQ	BLQ
3/29/2018	MB	0	BLQ	BLQ	BLQ
4/5/2018	MB	0	BLQ	BLQ	BLQ
4/18/2018	MB	0	BLQ	BLQ[3]	BLQ
4/18/2018	FB	Not performed	BLQ	0.96	BLQ
4/26/2018	MB	0	BLQ	BLQ	BLQ
5/2/2018	MB	10	BLQ	BLQ	BLQ
5/17/2018	MB	0	BLQ	BLQ	BLQ
5/24/2018	MB	0	BLQ	BLQ	BLQ

5/30/2018	MB	0	BLQ	BLQ	BLQ
5/30/2018	FB	0	BLQ	BLQ	BLQ

[1] MB = Method blank. FB = Field blank.
[2] BLQ = Below limit of quantification: <50 gene copies/L for *M. avium* and *M. intracellulare*; <100 gene copies/L for *L. pneumophila* serogroup 1.
[3] 1 of 3 replicates positive at a concentration of 0.86 gene copies/L; 2 of 3 replicates positive is considered a positive result.

Table A2. qPCR controls; Ct <40 is considered a positive result and are otherwise reported as Undetermined

Control	Plate #	*M. avium* (Ct)	*M. intracellulare* (Ct)	*L. pneumophila* serogroup 1 (Ct)
10^6 copies	1	22.72	18.65	17.71
10^5 copies	1	26.33	22.23	21.65
10^4 copies	1	30.01	25.81	24.93
10^3 copies	1	33.18	29.16	28.37
10^2 copies	1	35.98	32.69	31.99
10^1 copies	1	38.25	35.79	35.26
10^0 copies	1	Undetermined	Undetermined	Undetermined
NTC[1]	1	Undetermined	Undetermined	Undetermined
NTC	1	Undetermined	Undetermined	Undetermined
10^6 copies	2	22.71	18.70	17.79
10^5 copies	2	26.16	22.29	21.70
10^4 copies	2	29.99	25.71	25.10
10^3 copies	2	33.18	29.25	28.53
10^2 copies	2	36.32	32.66	32.27
10^1 copies	2	38.95	35.49	33.84
10^0 copies	2	Undetermined	Undetermined	38.83
NTC	2	Undetermined	Undetermined	Undetermined
NTC	2	Undetermined	Undetermined	Undetermined

[1] NTC = No template control.

Table A3. qPCR results for exogenous internal positive controls (average of 3 replicates); acceptable Ct range is 25.40–28.40 and Undetermined indicates a completely inhibited reaction

	Collection Date	*M. avium* (Ct)	*M. intracellulare* (Ct)	*L. pneumophila* serogroup 1 (Ct)
Raw Water	3/14/2018	26.78	26.78	26.65
	3/21/2018	26.80	26.80	26.59
	3/29/2018	26.62	26.62	26.56
	4/18/2018	26.77	26.77	26.50
	4/26/2018	26.75	26.75	26.51
	5/17/2018	26.86	26.86	26.64
	5/24/2018	26.92	26.92	26.65

	5/30/2018	26.63	26.63	26.54
Treated Water	3/14/2018	26.82	26.82	26.49
	3/21/2018	27.96	27.96	27.62
	3/29/2018	26.72	26.72	26.44
	4/5/2018	26.80	26.80	26.59
	4/18/2018	26.95	26.95	26.78
	4/26/2018	28.26	28.26	27.99
	5/2/2018	Undetermined	Undetermined	Undetermined
	5/17/2018	Undetermined	Undetermined	Undetermined
	5/24/2018	Undetermined	Undetermined	Undetermined
	5/30/2018	Undetermined	Undetermined	Undetermined

Table A4. Daily check standards for pH and conductivity measurements; acceptable ranges are 5.8–6.2 and 95–105 µS/cm, respectively

Collection Date	pH	Conductivity (µS/cm)
3/15/2018	6.01	97
3/19/2018	6.00	Not performed
3/26/2018	6.02	Not performed
4/2/2018	6.01	1000[1]
4/9/2018	5.99	104
4/17/2018	5.99	104
4/24/2018	6.04	105
4/30/2018	6.05	103
5/7/2018	8.01[2]	104
5/14/2018	7.95[2]	96
5/21/2018	6.02	105
5/29/2018	8.01[2]	104
6/4/2018	7.97[2]	104

[1] Substituted 1000 µS/cm standard due to exhausted reagent; acceptable range 995–1005 µS/cm.
[2] Substituted pH 8 standard due to sample pH noted >7; acceptable range 7.8–8.2.

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